

RESEARCH AND DEVELOPMENT, NEUCHATEL - QUARTERLY REPORT

DIVISION : RESEARCH
SUBJECT TITLE : TITANIA
PERIOD COVERED : OCTOBER - DECEMBER 1988
WRITTEN BY : Hofer-M. (MIH), Berney-J. (JBE)
KEYWORDS : bacillus, spore, germination, cell,
water-activity, ph, inhibition, activation,
heat-shock, cigarette, taste

OBJECTIVE

To study risks of physiological changes in the bacterial population during tobacco processing and storage, and to investigate their impact on the organoleptic and chemical properties of tobacco.

STATUS

The effect of the heat-shock on Bacillus spore germination was investigated as a function of water activity.

Cellular extracts of germinated spores were added to cut tobacco for analytical and subjective evaluation.

RESULTS

Germination activation

In order to establish the impact of tobacco processing on Bacillus spore germination, the effects of heat-shock in combination with water activity (*aw*) were evaluated in simulation trials. Simulation media were prepared from tobacco extract supplemented with glycerol to obtain *aw* values ranging between 0.77 and 1.0 [1]. Spore suspensions were heat-shocked at 60, 70, 80, 90 and 100°C for 30 min. and cooled rapidly prior to the incubation in tobacco medium. Germination was followed over time by measuring the loss in absorbance at 660 nm [2].

Fig. 1 shows the effects of heat-shock temperature on spore germination for *aw* = 1.0. At 90 and 100°C no germination could be observed. Compared to control the trials activated at 60, 70

2028635279

RESEARCH AND DEVELOPMENT, NEUCHATEL - QUARTERLY REPORT

and 80°C show no lag phase at the beginning of germination. Completion of germination decreases by increasing activation temperature. After 90 min., 100% of the spore population had germinated at 60°C, 90% at 70°C and only 75% at 80°C. The germination rates follow the same trend. At 60 and 70°C the maximum rate measured was 2%/min. and at 80°C 1%/min. A germination rate of only 0.5%/min. was found in the control.

Fig. 2 shows combined effects of heat-shock and water activity after 120 min. incubation for five different aw values. In the range of 0.9-1.0, spore germination is clearly activated by a heat-shock between 60 and 70°C compared to an unheated control. The version treated at 80°C shows the same profile as the control. At 90°C a slight germination (10%) was observed for aw values of 0.9, but no germination was recorded for the activation at 100°C. The germination activation created by a heat-shock between 60 and 70°C and a water activity of between 0.9 and 1.0 corresponds to situations encountered during tobacco processing.

Germination and off-taste

A relation between *Bacillus* spore germination and a taste change in cigarettes was found [3]. It was assumed that activation conditions created in the dryer allowed spores to germinate and that germinated cells unable to outgrow on tobacco due to low aw are osmolyzed. In order to verify this implication, a *B. pumilus* spore preparation was allowed to germinate, germinated cells were broken and cellular extract was injected into cigarettes for subjective evaluation. Two versions were prepared : one with the equivalent of the tobacco microflora (i.e. 10⁶ cells/g) and the other with ten times more cell extract. After conditioning, the two versions, as well as a water treated control were evaluated vs an untreated control by an in-house panel.

Version	Comments	Preference
untreated control	standard quality	1
control + water	idem control	1
+ 1 cell. equiv.	less flavor sensations, slight dark side	3
+ 10 cell. equiv.	after taste, cover the mouth, dark side	4

Preferences : 1 = best 4 = worst

Both controls were in line with the standard quality. An increase in taste change was recorded with increasing cellular extract concentration. The main line in the taste description concerns a deviation to the dark side. This dark note was also described by an expert panel for off-taste cigarettes from project PLEIADE [3,4].

2028635280

RESEARCH AND DEVELOPMENT, NEUCHATEL - QUARTERLY REPORT

CONCLUSIONS

=====

Heat-shock temperatures of 60 and 70°C in the aw range of 0.9-1.0 considerably accelerate *Bacillus pumilus* spore germination.

Cellular extract of germinated spores added to cut filler causes off-taste in cigarettes.

PLANS

=====

- Study germination activation by heat-shock and aw during Burley and cut tobacco treatments.
- Produce at mini-primary scale a cut filler sprayed with cellular extract of germinated spores for subjective and chemical evaluation.
- Verify the impact of cellular extract on cigarette taste by panel A evaluation and establish a correlation with known off-taste problems.
- Collaborate on chemical investigations on cellular extract composition and the possible interactions with the tobacco matrix.

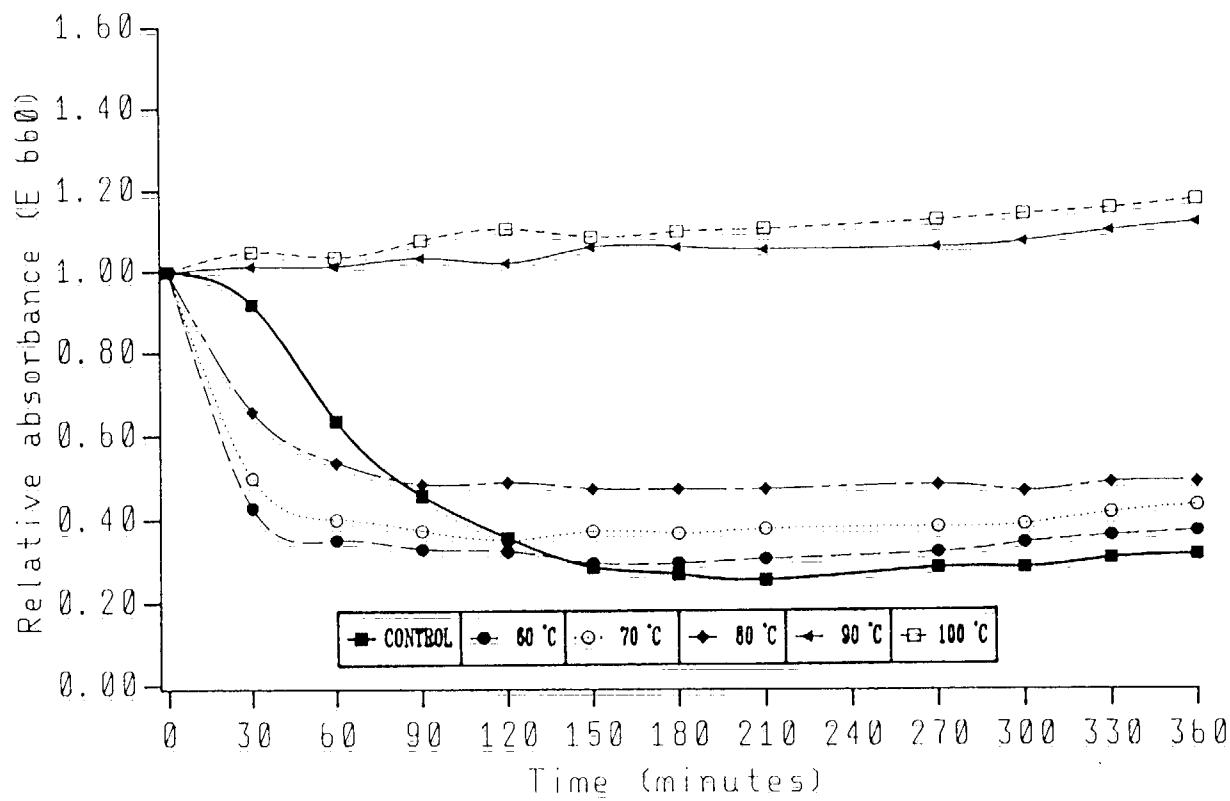
REFERENCES

=====

- [1] Hofer-M., Kälin-P., Quarterly Report, EUROP, January-March 1988.
- [2] Kälin-P., Quarterly Report, EUROP, April-June 1988.
- [3] Memo from Hofer-M. to Fink-W., Project PLEIADE I, July 14, 1988.
- [4] Memo from Hofer-M. to Fink-W., Project PLEIADE II, November 22, 1988.

2028635281

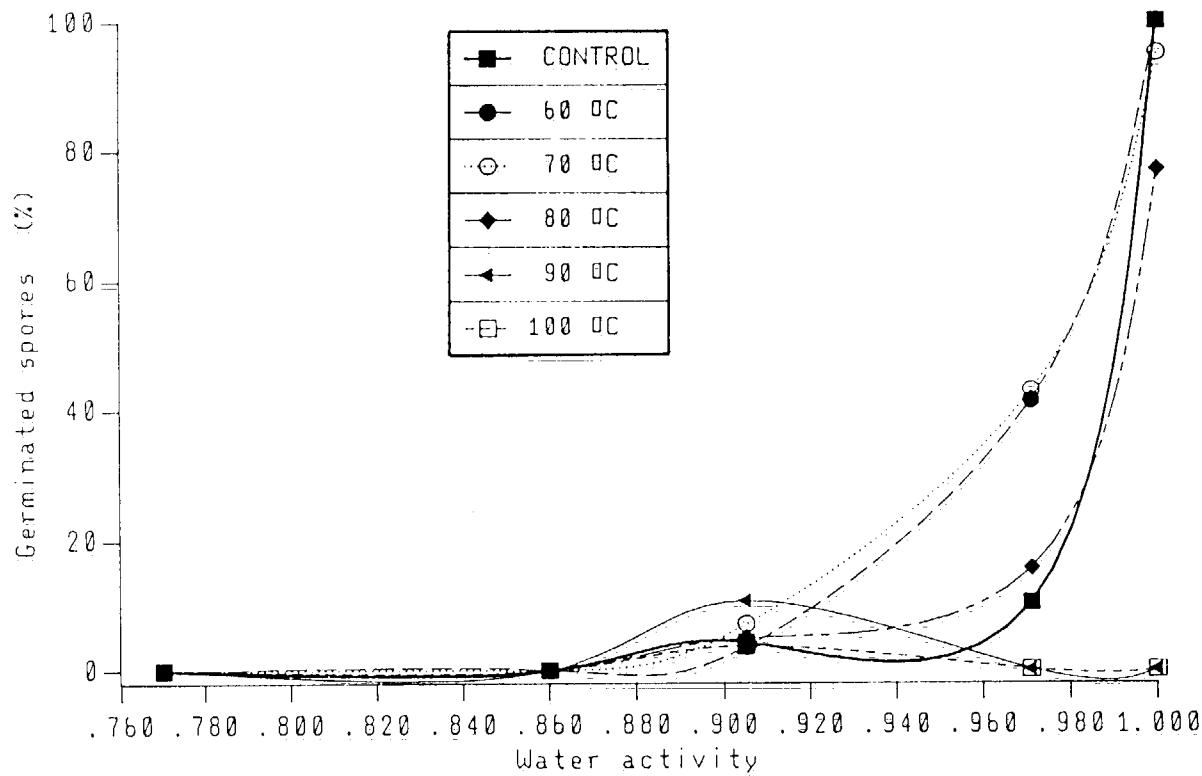
Figure 1 : EFFECT OF HEAT-SHOCK ON SPORE GERMINATION
AW = 1



2028635282

Figure 2 :

EFFECT OF HEAT-SHOCK ON SPORE GERMINATION AS A FUNCTION OF WATER ACTIVITY



2028635283